AMENDMENTS TO THE SPECIFICATION

At page 1, line 1, immediately after the title, please insert the following:

CROSS-REFERENCE TO RELATED APPLICATIONS

This patent application is the U.S. national phase of International Patent Application PCT/JP04/09785, filed on July 2, 2004, which claims the benefit of Japanese Patent Application No. 2003-191081, filed on July 3, 2003.

Please replace the paragraph at page 1, lines 20-28, with the following:

Conventionally, proteins directly extracted from living body components have been often used as blocking agents used for immunoassays. In particular historically, albumin and casein derived from bovine have been widely used. However, recently, various limitations have occurred owing to problems such as mad ~~eaw~~ cow disease. Meanwhile, method of producing them using recombinant technology has an advantage that a pathogen (substance) can be excluded, but is virtually off from practical use due to problems such as productivity.

Please replace the paragraph at page 5, lines 13-15, with the following:

(2) a step of adding normal human serum diluted 25 to 100 times with PBS(-), leaving stand at 37°C for one hours, and subsequently washing the plate with PBS(-) (0.05% ~~Tween~~ TWEEN® (polysorbate) 20); and

Please replace the paragraph at page 5, lines 27-28, with the following:

(3) a step of leaving stand at 25°C for one hour, removing the solutions and washing with PBS(-) containing 0.02% ~~Tween~~ TWEEN® (polysorbate) 20;

Please replace the paragraphs at page 6, lines 11-15, with the following:

(2) a step of adding a peroxidase solution prepared at 0.05 mg/mL, leaving stand at 37°C for one hour and subsequently washing the plate with PBS(-) (0.05% ~~Tween~~ TWEEN® (polysorbate) 20);

(3) a step of leaving stand at 25°C for one hour, subsequently removing the solution and washing with PBS(-) containing 0.02% ~~Tween~~ TWEEN® (polysorbate) 20; and

Please replace the paragraph at page 18, lines 23-25, with the following:

2. A step of adding normal human serum diluted 25 to 100 times with PBS(-), leaving stand at 37°C for one hours, and subsequently washing the plate with PBS(-) (0.05% ~~Tween~~ TWEEN® (polysorbate) 20).

Please replace the paragraph at page 19, lines 13-27, with the following:

The human serum used herein is not particularly limited as long as it is the serum isolated from a normal adult. Since the undiluted serum is at high concentration, it is better to dilute 25 to 50 times with PBS to use. Since the amount of serum IgG is different in individuals, a dilution rate may be slightly changed. The non-specific absorption to the plate is performed at 37°C for 30 minutes. In order to precisely measure, it is preferable that the amount of the serum added at this time is smaller than that of the solution used for the first blocking. For example, when 100 μL is used for the blocking, 50 μL of a diluted serum solution is added. Finally, after reacting precisely for one hour, the solution is removed, and the plate is washed with the sufficient amount of 0.05% ~~Tween~~ TWEEN® (polysorbate) 20/PBS(-). It is preferable to wash three times, and more preferably four times. Washing may be performed using a plate washer for exclusive use.

Please replace the paragraph at page 26, line 8, through page 27, line 16, with the following:

The present invention also defines the blocking speed. That is, the present invention is the modified protein characterized in that the blocking speed is enhanced compared with that of BSA. Particularly, the present invention is also the modified protein characterized in that the blocking ability for less than 10 minutes is more excellent than that of BSA under the condition where the protein amounts have been adjusted so that the blocking efficiency equivalent to that of BSA is exhibited in blocking for 3 hours. The modification herein indicates that a gene sequence encoding the protein of a wild type has been converted by amino acid substitution, deletion and insertion. An evaluation method is not particularly limited, methods shown in Examples 5 and 7 are preferably used, and the blocking is measured using non-specific absorption of IgG or peroxidase to the polystyrene plate as the indicator. In the evaluation method using IgG, the blocking is performed for 1 to 10 minutes, preferably 2 to 10 minutes, subsequently human serum diluted with PBS(-) is added, then washing is performed, an anti-human IgG antibody at an optimal concentration is reacted, subsequently washing is performed, and then the amount of non-specifically absorbed IgG is measured by absorbance of colored products from TMBZ. Also in the evaluation method using peroxidase, first, horseradish peroxidase for labeling (supplied from Toyobo Co., Ltd., PEO-131) is dissolved at 2 mg/mL in the protein solution whose blocking ability is to be measured. Then serial dilutions of 40 to 320 times of the peroxidase solutions are made from the above solution, and 100 μL of each dilution is dispensed in a polystyrene 96-well microplate. The plate is left stand at room temperature for one hour, subsequently the solution is removed, and the plate is washed with PBS buffer containing 0.02% ~~Tween~~ TWEEN® (polysorbate) 20. This washing manipulation is repeated six times, and then the washing solution is thoroughly removed. Tetramethylbenzidine solution is added, the plate is incubated at 37°C precisely for 10 minutes, and then 1N sulfuric acid is added to stop the reaction and develop the color. This absorbance is measured by a microplate reader at a major wavelength of 450 nm and minor wavelength of 650 nm. Details will be described in Example 7. Likewise, the diluted peroxidase solution may be added to the solid phase previously blocked, and its non-specific absorption may be measured. The concentration of the peroxidase solution used herein is preferably 0.05 mg/mL, but it conductive to the correct

evaluation to control and use so as to measure in the commonsense range. The dilution of peroxidase is preferably performed using PBS(-), but is not particularly limited.

Please replace the paragraph at page 35, lines 6-29, with the following:

As the method, 100 μL of BSA (Fragment V, supplied from Sigma) and various DnaK samples diluted with 20 mM Tris-HCl (pH 7.0) were added to a polystyrene 96-well immunoplate (E.I.A./R.I.A. 8 Well Strip, supplied from Costar), left stand at 4°C for 4 hours (basically the plate was left stand for 4 hours, but when blocking time periods were examined, optional time periods were set.). Subsequently, the solution was removed from the plate, then 50 μL of normal human serum diluted with PBS(-) 50 times was added, and incubated at 37°C for one hour. Subsequently, each well was washed four times with 200 μL of a washing solution (PBS(-), 0.05% ~~Tween~~ TWEEN® (polysorbate) 20), 50 μL of a peroxidase-labeled anti-human IgG antibody (supplied from Jackson ImmunoResearch) diluted with an antibody dilution (0.01 M PB (pH 7.4), 0.15 M NaCl, 0.5% casein) to an optimal concentration was added to each well, and incubated at 37°C for one hour. Subsequently, each well was washed four times with 200 μL of the washing solution (PBS(-), 0.05% ~~Tween~~ TWEEN® (polysorbate) 20), 100 μL of a color development reagent (3,3',5,5'-tetramethylbenzidine, TMBZ) was added to allow color development at room temperature for 5 minutes, and then 50 μL of 1N sulfuric acid was added to stop the reaction. The color development was measured using an ELISA reader at a major wavelength of 450 nm and a minor wavelength of 650 nm. The Bradford method was used for quantification of the protein.

Please replace the paragraph at page 33, line 35, through page 34, line 24, with the following:

Usefulness of the DnaK fragments as the blocking agent was examined using an ELISA system for human carcinoembryonic antigen (hCEA). First, an anti-hCEA MoAb was diluted to 10 μL/mL with 50 mM carbonate buffer (pH 9.6), 100 μL of aliquot was added to a

polystyrene immunoplate (E.I.A./R.I.A. 8 Well Strip, supplied from Costar), and then left stand at 37°C for one hour. After leaving stand, each well was washed three times with 150 μL of the washing solution (PBS(-), 0.05% ~~Tween~~ TWEEN® (polysorbate) 20), subsequently 200 μL of a blocking solution was added, and left stand at 4°C for 4 hours. As the blocking solution, 2.4 mg of BSA (20 mM Tris-HCl, pH 7.0) generally used and DnaK 419-607 dissolved in the same buffer were used, and 20 mM Tris-HCl (pH 7.0) alone was added as a blank. After removing the blocking solution, 50 μL of an hCEA solution (Immunoflora supplied from Toyobo Co., Ltd.) diluted to 0, 2.5 ng/mL and 5 ng/mL was added, the plate was incubated at 37°C for one hour, and then washed four times with 150 μL of the washing solution. Subsequently, a peroxidase-labeled anti-hCEA antibody (Immunoflora supplied from Toyobo Co., Ltd.) diluted to an optimal concentration was added, reacted at 37°C for one hour, and the plate was washed three times with 150 μL of the washing solution (PBS(-), 0.05% ~~Tween~~ TWEEN® (polysorbate) 20). Then, 100 μL of a substrate solution (3,3',5,5'-tetramethylbenzidine, TMBZ) was added to develop a color at 37°C for 20 minutes with shielding light. Finally, 100 μL of a reaction stop solution (1N H_2SO_4) was added, and the developed yellow color was measured at 450 nm/650 nm.

Please replace the paragraph at page 35, line 21, through page 36, line 4, with the following:

That is, *Escherichia coli* JM109 (pQE-DnaK 419-607N) was inoculated to terrific broth (1.2% polypeptone, 2.4% yeast extract, 0.5% glycerol, 17 mM monopotassium phosphate, 72 mM dipotassium phosphate) containing 100 mg/mL of ampicillin, and cultured at 32°C for 20 hours with shaking. Microbial cells corresponding to 1 L of this culture were collected by centrifugation, suspended in 200 mL of 100 mM Tris-HCl buffer pH 9.0, and disrupted by French press. Polyethylene imine was added at a final concentration of 0.1% to this disruption solution, heated at 60°C for 2 hours, and the supernatant was collected by centrifugation. Then, ammonium sulfate with 50% saturation was added, a precipitate was collected by centrifugation, and re-dissolved in 100 mM Tris-HCl buffer, pH 9.0. Furthermore, the solution was heated at 64°C for 14 hours, and the supernatant was collected by centrifugation. This crude enzyme solution was subjected to ~~Superdex~~ SUPERDEX® 200

(supplied from Amersham Bioscience) gel filtration chromatography, then ammonium sulfate with 50% saturation was added, the precipitate was collected by centrifugation, and re-dissolved in 100 mM Tris-HCl buffer, pH 9.0. The solution was desalted by gel filtration chromatography using ~~Sephadex~~ SEPHADEX® G-25 (supplied from Amersham Bioscience) equilibrated with the same buffer. Subsequently, this DnaK fragment was subjected to the column chromatography using ~~Phenyl Sepharose~~ PHENYL SEPHAROSE™ Fast Flow equilibrated with 100 mM Tris-HCl buffer pH 9.0 containing 20% saturated ammonium sulfate, and eluted with a linear gradient of 20 to 0% of saturated ammonium sulfate to acquire a purified DnaK fragment fraction. This purified fragment fraction was condensed by ultrafiltration, and desalted with distilled water to acquire the finally purified DnaK fragment.

Please replace the paragraph at page 36, lines 17-35, with the following:

First, horseradish peroxidase (PEO-131, supplied from Toyobo Co., Ltd.) for labeling was dissolved at 2 mg/mL in PBS buffer containing BSA or the native DnaK 419-607 fragment. At that time, commercially available BSA (fraction V) was prepared at the concentration of 2 or 10 mg/mL, and the native DnaK 419-607 fragment was prepared at the concentration of 0.1 or 0.5 mg/mL. Then, serial dilutions of 40 to 320 times of the peroxidase dissolution were made using the same solutions, and 100 μL of each dilution was dispensed to a polystyrene 96-well microplate. The plate was left stand at room temperature for one hour, then the solution was removed, and the well was washed with 200 μL of PBS buffer containing 0.02% ~~Tween~~ TWEEN® (polysorbate) 20. This washing manipulation was repeated six times, and subsequently the washing solution was thoroughly removed. Then, 100 μL of a tetramethylbenzidine solution (supplied from Bio-Rad) was added, incubated at 37°C precisely for 10 minutes, and 100 μL of 1N sulfuric acid was added to stop the reaction and develop the color. This developed color was measured by a microplate reader at a major wavelength of 450 nm and a minor wavelength of 650 nm.